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Alterations of small-molecular-weight antioxidants in the blood of smokers

Chin San Liu [a,b,c], Huei Wen Chen [d], Chong Kuei Lii [d], Su Chiu Chen [e], Yau-Huei Wei [a,f,*]

[a] *Institute of Clinical Medicine, School of Medicine, National Yang-Ming University, Taipei, 112, Taiwan, ROC*

[b] *Department of Neurology, Taichung Municipal Aging Care Hospital, Taichung, 400, Taiwan, ROC*

[c] *Department of Neurology, Kuang Tien General Hospital, Taichung, 430, Taiwan, ROC*

[d] *Department of Nutrition, Chung Shan Medical College, Taichung, 402, Taiwan, ROC*

[e] *Department of Health and Nursing Service Administration, National Taipei College of Nursing, Taipei, 108, Taiwan, ROC*

[f] *Department of Biochemistry and Center for Cellular and Molecular Biology, National Yang-Ming University, Taipei, 112, Taiwan, ROC*

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Abstract

Plasma α-tocopherol, ascorbate, retinol, uric acid, and lipid peroxides were investigated in 39 male smokers and 64 male non-smokers. The average level of plasma α-tocopherol of 35–45-year-old smokers (1.74 ± 0.49 μg/mg total lipid) was significantly lower than that of age-matched non-smokers (2.55 ± 0.88 μg/mg total lipid, $P = 0.032$). Similarly, the plasma α-tocopherol of smokers aged above 45 (1.66 ± 0.29 μg/mg total lipid) was lower than that of the age-matched non-smokers (2.38 ± 1.26 μg/mg total lipid, $P = 0.014$). However, no difference in plasma level of α-tocopherol was found between smokers and non-smokers below the age of 35. The average concentration of ascorbate in plasma was significantly decreased only in those smokers older than 45 (0.33 ± 0.16 mg/dl vs non-smokers 0.53 ± 0.19 mg/dl, $P = 0.003$). The average level of lipid peroxides (measured as malondialdehyde,

* Corresponding author. Department of Biochemistry, School of Life Science, National Yang-Ming University, Taipei 112, Taiwan, ROC. Tel.: +886 2 28267118; fax: +886 2 28264843; e-mail: joeman@mailsrv.ym.edu.tw

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MDA) in the plasma of smokers (2.77 ± 0.51 nmol/ml) was higher than that of non-smokers (2.35 ± 0.21 nmol/ml) aged above 45 ($P = 0.034$). No differences in the plasma levels of uric acid and retinol were noted between smokers and non-smokers in all age groups. Using partial correlation analysis under age control, we found that the plasma level of α-tocopherol was negatively correlated with the plasma level of MDA ($r = -0.523$, $P = 0.038$). In contrast, the plasma level of ascorbate was only weakly correlated with the plasma level of MDA ($r = -0.341$, $P = 0.094$). Moreover, we found a negative correlation between the plasma level of α-tocopherol and smoking index ($r = -0.414$, $P = 0.006$) under age control, but there was no correlation between plasma level of ascorbate and smoking index ($r = 0.221$, $P = 0.193$). These results indicate that adequate levels of α-tocopherol and ascorbate may protect the plasma from oxidative damage elicited by smoking-mediated reactive oxygen species (ROS) and free radicals in young smokers. However, the antioxidant activities of α-tocopherol and ascorbate may be overwhelmed by the long-standing oxidative stress elicited by cigarette smoking in elderly subjects. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Smoking; Aging; α-Tocopherol; Retinol; Ascorbate; Uric acid; Lipid peroxidation

1. Introduction

Pryor and Stone [1] reported that gas-phase cigarette smoke contains 1×10^{15} organic radicals per puff, which are primarily of the alkyl, alkoxyl, and peroxyl types. These chemically reactive species are known to be present or formed in cigarette smoke which may lead to the modification of biological macromolecules [2]. The relatively high levels of these free radicals in cigarette smoke can deplete antioxidants and initiate peroxidation of unsaturated fatty acids and modification of proteins and nucleic acids in biological systems [3,4]. Aside from smoke-borne organic radicals, cigarette smoking can also induce endogenous production of reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide, and hydroxyl radicals, and thereby increases intracellular oxidative stress in various tissues.

α-Tocopherol, ascorbate, retinol, and uric acid are four important small-molecular-weight antioxidants involved in the defense of tissue cells against damage induced by smoking-mediated ROS and free radicals. α-Tocopherol is a fat-soluble nutritional factor, and can trap organic free radicals and protect proteins and lipids in biological membranes from oxidative damage [5,6]. Ascorbate is a water-soluble vitamin able to act as a strong reducing agent, which is required for detoxification of various xenobiotics-derived organic radicals in vivo. Frei et al. [7] demonstrated that ascorbate is the most effective water-soluble antioxidant in human blood plasma and suggested that ascorbate is one of the major physiological antioxidants essential for the protection of the human against chronic diseases and degenerative disorders caused by oxidative stress. In

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addition, ascorbate is an effective scavenger of aqueous peroxyl radicals in plasma and normally provides a significant health benefit at a certain concentration range [3]. Ascorbate has also been shown to be an anti-carcinogen in rodents treated with benzo[*a*]pyrene and nitrite, which are the major toxic compounds in tobacco smoke [7]. Therefore, α-tocopherol and ascorbate play important roles in the defense against the toxicity of ROS and xenobiotics. In addition to ascorbate and α-tocopherol, uric acid and retinol have also been demonstrated to be potent antioxidants [8,9]. Uric acid is a powerful scavenger of singlet oxygen, peroxyl radicals, and hydroxyl radicals, which suggests that uric acid may function as an antioxidant in vivo [8]. Retinol supplementation to smoke-exposed rats was found to increase the resistance of the rats to lipid peroxidation and increase the activities of free radical scavenging enzymes [9].

Most of the smoking-mediated free radicals and ROS are converted to ultimate prooxidants in the lung and blood circulation. Therefore, these smoking-mediated free radicals may cause alterations of the levels of the aforementioned four small-molecular-weight antioxidants before they react with negatively charged centers in the intracellular macromolecules, such as nuclear DNA or mitochondrial DNA (mtDNA) of the target tissues. In previous studies, we found significant alterations in the concentrations of blood thiol-group-related antioxidants and accumulation of mtDNA mutations in the hair follicles of aging smokers [10,11]. Thus, we have further investigated whether alterations in the levels of the small-molecular-weight antioxidants in blood circulation are involved in smoking-induced increases of oxidative stress and oxidative damage in the human.

2. Materials and methods

2.1. Subjects and biochemical analysis

A total of 103 healthy males including 64 non-smokers and 39 smokers, who had no systemic diseases and did not take vitamin supplements, were recruited in this study through a special clinic in Kuang Tien General Hospital, Taichung, Taiwan. Ex-smokers or subjects with discontinuous smoking were excluded from both the smokers' group and the non-smokers' group. The smoking index was defined as the number of cigarettes smoked per day × years of smoking. Blood samples were taken with consent from each of the study subjects in the morning after overnight fasting before any medication or smoking. From each subject 10 ml of whole blood was withdrawn from an antecubital vein and transferred to a heparin-containing blood sampling tube. Plasma was immediately collected by centrifugation and divided into several aliquots and stored at −80°C in metal-free plastic tubes. The concentrations of the small-molecular-weight antioxidants and lipid peroxides in the plasma were determined within 3 days, but the concentration of ascorbate was determined within 24 h. The general data of the study subjects are summarized in Table 1.

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Table 1
General data of the male non-smokers and smokers in different age groups

	Age < 35			35 ≥ Age ≤ 45			Age > 45		
	Non-smokers[a] ($n = 19$)	Smokers ($n = 16$)	P-value	Non-smokers ($n = 38$)	Smokers ($n = 21$)	P value	Non-smokers ($n = 14$)	Smokers ($n = 10$)	P value
Age	30 ± 4.6	33 ± 1.9	0.078[e]	40 ± 2.9	42 ± 2.2	0.052[e]	53 ± 7.9	59 ± 9.3	0.238[e]
BMI[b]	23 ± 3.5	25 ± 2.5	0.203[e]	24 ± 3.4	24 ± 3.2	0.486[e]	25 ± 3.0	23 ± 3.0	0.174[e]
Fruits (U[c]/day)	2.5 ± 1.5	2.5 ± 2.4	0.561[e]	2.8 ± 2.0	2.0 ± 1.8	0.072[e]	3.1 ± 2.5	2.8 ± 1.1	0.682[e]
Leafy vegetables (U[c]/day)	2.0 ± 0.9	1.6 ± 0.8	0.098[e]	2.0 ± 1.1	1.1 ± 0.9	0.004[e,f]	2.0 ± 1.8	1.8 ± 1.1	0.907[e]
Plasma total lipid (mg/dl)	387 ± 105	456 ± 123	0.136[e]	380 ± 95	443 ± 81	0.035[e,f]	530 ± 259	447 ± 117	0.815[e]
Smoking index[d]	0	139 ± 170		0	457 ± 249		0	473 ± 316	

[a] Arithmetic mean ± S.D.
[b] Body mass index.
[c] Unit defined by the Taiwan Provincial Sanitary Station of the Republic of China.
[d] Number of cigarettes smoked per day × years of smoking.
[e] Comparison by Mann–Whitney U test (2-tailed).
[f] $P < 0.05$.

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2.2. Determination of plasma levels of α-tocopherol and retinol

The α-tocopherol and retinol concentrations in blood plasma were determined using a procedure modified from that described by Catignani and Bieri [12]. An aliquot of 50 μl of internal standards (1.2 mg/l retinyl acetate and 52.5 mg/l α-tocopheryl acetate in ethanol) and 100 μl of plasma was mixed by vortexing for 1 min. For extraction of lipid, 200 μl HPLC-grade *n*-hexane was added and mixed for another 1 min. The organic phase was separated from the aqueous phase by centrifugation at 800 × *g* for 2 min. The upper *n*-hexane layer was withdrawn and evaporated under a stream of nitrogen. The residue was re-dissolved in 50 μl of HPLC-grade methanol and an aliquot of 20 μl was injected into an HPLC system for chromatographic analysis of retinol and α-tocopherol at ambient temperature. The HPLC system consisted of an L-6200A intelligent pump, an L-4200 UV-visible detector, a model D-6000 interface, and an LC organizer (Hitachi, Tokyo, Japan). The sample was chromatographed on a μBondapak C_{18} column (3.9 mm × 30 cm stainless steel), which had been connected to a guard column of 3 × 22 mm that was also packed with the μBondapak C_{18} matrix. The α-tocopherol and retinol in the eluate were respectively monitored with a UV detector set at the wavelength of 290 nm and a sensitivity of 0.01 absorbance unit full scale. The mobile phase was 98% HPLC-grade methanol, and the flow rate was 1.2 ml/min. Peak area ratios of the samples were converted to μg α-tocopherol and μg retinol by using the standard curves constructed with the aforementioned internal standards, respectively. Total plasma lipid concentration was determined and used to adjust the value of α-tocopherol [13], which is thus expressed as μg/mg total lipid.

2.3. Determination of plasma levels of ascorbate

The concentration of plasma ascorbate was measured by a method described by Kyaw et al. [14]. To 0.5 ml of fresh plasma in a centrifuge tube, we slowly added 1.0 ml of the color reagent containing 0.3 mM sodium tungstate, 1.2 mM disodium hydrogen phosphate and concentrated sulfuric acid. After thoroughly and slowly mixing, the mixture was allowed to stand for 30 min at room temperature, and then centrifuged at 1000 × *g* for 15 min. The blue-colored supernatant was transferred to another test tube with a Pasteur pipette without disturbing the precipitate. Absorbance at 700 nm was read against a reagent blank. For every set of experiments, a standard and a blank were run in parallel throughout the procedure. Plasma level of ascorbate is expressed as mg/dl.

2.4. Determination of plasma level of uric acid

A commercial kit (Sigma, Dorset, England) was used to determine the concentration of uric acid in the plasma. Briefly, uricase catalyzes the oxidation of uric acid to generate allantoin, CO_2 and H_2O_2. In the presence of peroxidase, 4-aminoantipyrine and 3,5-dichloro-2'-2'-hydroxybenzenesulfonate, H_2O_2 induces the formation of a

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quinoneimine complex with an absorbance maximum at 520 nm. The intensity of the pink color of the product is proportional to the concentration of uric acid in the plasma sample. The concentration of uric acid is expressed as mg/dl.

2.5. Measurement of plasma level of lipid peroxides

The concentration of lipid peroxides in blood plasma was measured as malondialdehyde (MDA) using a method modified from the protocol described by Dousseet et al. [15]. Blood was withdrawn into a heparinized tube containing 10 μl of 2 mM butylated hydroxytoluene, and the blood sample was immediately centrifuged to obtain plasma. An aliquot of 20 μl plasma was mixed with 2 ml of 0.1 N HCl, 0.3 ml of 10% phosphotungstic acid, and 1 ml of 0.7% thiobarbituric acid (TBA). The mixture was heated for 60 min in boiling water, followed by extraction of the MDA-TBA adduct with 5 ml 1-butanol. After centrifugation, the fluorescence intensity of the MDA-TBA complex in the 1-butanol layer was quickly measured in a fluorescence spectrophotometer using the excitation wavelength of 525 nm and emission wavelength of 550 nm. The concentration of lipid peroxides in the plasma is expressed as nmol MDA/ml. A calibration curve was constructed for each run by using 1,1,3,3-tetraethoxypropane as the standard.

2.6. Statistical analysis

Mann-Whitney *U* test and multivariate ANOVA analysis were applied in the analysis of data with non-normal and normal distributions, respectively. Partial correlation analysis was also applied in the search for a causal relationship between two parameters under age control. All statistical analyses were performed using the SPSS statistical package. The difference between two measured parameters with a *P*-value smaller than 0.05 is considered significant.

3. Results

There were no differences in age, body mass index (BMI), total plasma lipid, or dietary intake of fruits and vegetables between smokers and non-smokers in all three age groups (Table 1). However, a significant increase in plasma lipid and a decrease in dietary intake of vegetables were noted in smokers aged 35–45 years. As shown in Table 2, there were no significant differences in the plasma levels of α-tocopherol/total lipid and ascorbate between smokers and non-smokers below the age of 35. However, the average level of plasma α-tocopherol of smokers (1.74 ± 0.49 μg/mg total lipid) was significantly lower than that of non-smokers (2.55 ± 0.88 μg/mg total lipid, $P = 0.032$) in the 35–45-year-old age group. Similarly, the plasma α-tocopherol of smokers aged above 45 (1.66 ± 0.29 μg/mg total lipid) was lower than that of the age-matched non-smokers (2.38 ± 1.26 μg/mg total lipid, $P = 0.014$).

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A significantly lower level of plasma ascorbate was only observed in smokers older than 45 (0.33 ± 0.16 mg/dl vs non-smokers 0.53 ± 0.19 mg/dl, $P = 0.003$) (Table 2). On the other hand, the average level of plasma MDA of smokers (2.77 ± 0.51 nmol/ml) was significantly higher than that of non-smokers (2.35 ± 0.21 nmol/ml, $P = 0.034$) aged above 45 (Table 2). However, we found no difference in plasma MDA between smokers and non-smokers aged below 45.

Plasma levels of uric acid and retinol of smokers in all three age groups were not different from those of the age-matched non-smokers (Table 2). By partial correlation analyses of the measured parameters under age control, we found that the plasma level of α-tocopherol was significantly correlated in a negative manner with the level of plasma MDA ($r = -0.523$, $P = 0.038$) of all the study subjects (Fig. 1). Moreover, the plasma level of ascorbate was negatively correlated, although without statistical significance, with the level of plasma MDA ($r = -0.341$, $P = 0.094$) of all the study subjects (Fig. 2). In contrast, the plasma level of uric acid exhibited no significant correlation with the level of plasma MDA ($r = -0.132$, $P = 0.348$).

Table 2
The concentrations of small-molecular-weight antioxidants and lipid peroxides in the plasma of male smokers and non-smokers in different age groups[a]

		Non-smoker[a]	Smoker[a]	P-value
Age < 35		n = 19	n = 16	
	α-Tocopherol (μg/mg total lipid)	1.64 ± 0.39	1.55 ± 0.43	0.544[c]
	Retinol (μg/ml)	0.47 ± 0.11	0.43 ± 0.15	0.462[b]
	Ascorbate (mg/dl)	0.49 ± 0.11	0.44 ± 0.22	0.497[b]
	Uric acid (mg/dl)	5.29 ± 1.91	6.90 ± 1.46	0.098[b]
	Malondialdehyde (nmol/ml)	2.40 ± 0.30	2.53 ± 0.29	0.238[b]
35 ≥ Age ≤ 45		n = 38	n = 21	
	α-Tocopherol (μg/mg total lipid)	2.55 ± 0.88	1.74 ± 0.49↓	0.032[c,*]
	Retinol (μg/ml)	0.42 ± 0.18	0.44 ± 0.13	0.573[b]
	Ascorbate (mg/dl)	0.41 ± 0.13	0.45 ± 0.28	0.137[b]
	Uric acid (mg/dl)	6.55 ± 3.03	5.88 ± 0.92	0.558[b]
	Malondialdehyde (nmol/ml)	2.36 ± 0.40	2.33 ± 0.50	0.704[b]
Age > 45		n = 14	n = 10	
	α-Tocopherol (μg/mg total lipid)	2.38 ± 1.26	1.66 ± 0.29↓	0.014[c,*]
	Retinol (μg/ml)	0.40 ± 0.17	0.48 ± 0.15	0.341[b]
	Ascorbate (mg/dl)	0.53 ± 0.19	0.33 ± 0.16↓	0.003[c,*]
	Uric acid (mg/dl)	4.99 ± 1.21	5.39 ± 1.13	0.472[b]
	Malondialdehyde (nmol/ml)	2.35 ± 0.21	2.77 ± 0.51↑	0.034[b,*]

[a] Arithmetic mean ± S.D.
[b] Comparison by Mann–Whitney *U* test (2-tailed).
[c] Comparison by the multivariate ANOVA and control with total plasma lipid and dietary intake of vegetables.
* $P < 0.05$.
↑ Significant increase.
↓ Significant decrease.

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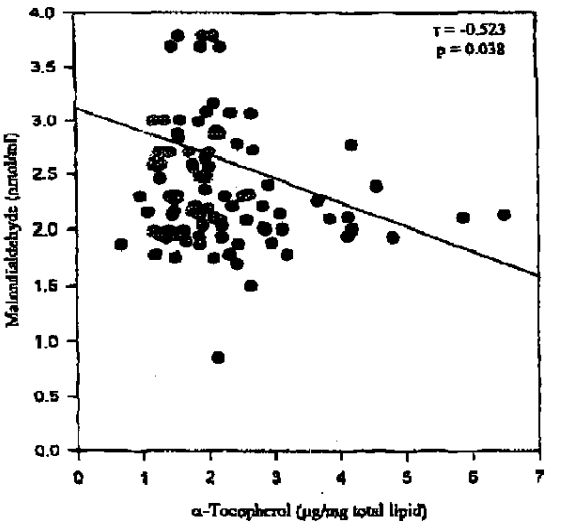

Fig. 1. Plasma level of malondialdehyde is negatively correlated with the level of plasma α-tocopherol. There are 103 data sets from 39 male smokers and 64 male non-smokers in the scattered plot. The *P*-value was calculated by partial correlation analysis under age control. *r*, sample correlation coefficient.

Partial correlation analysis of the plasma levels of the four small-molecular-weight antioxidants and the smoking index revealed a negative correlation between the plasma level of α-tocopherol and smoking index ($r = -0.414$, $P = 0.006$) under age control (Fig. 3). However, the correlation between the plasma level of ascorbate and smoking index was not statistically significant ($r = -0.221$, $P = 0.193$). There was no correlation at all between smoking index and the plasma levels of retinol or uric acid.

4. Discussion

Cigarette smoke contains large amounts of prooxidants and may induce the generation of ROS and free radicals in tissue cells. These smoke-induced reactive substances cause a depletion of extra- and intra-cellular antioxidants and may elicit oxidative damage to various cellular macromolecules. As reported by Pacht et al. [16], α-tocopherol level in the alveolar fluid of smokers is significantly lower than in non-smokers. Therefore, α-tocopherol supplementation is probably valuable for the protection of lower respiratory tract against smoking-associated oxidative damage [17]. However, Marangon et al. [18] reported that smoking did not exert significant effect on the serum concentration of α-tocopherol. In addition, Pons et al. [19] found that the level of plasma ascorbate was decreased in male smokers. Moreover,

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ascorbate in urinary excretion was also found be decreased in smokers [20]. Albanese et al. [21] showed that the concentration of ascorbate in the plasma and the number of leukocytes of smokers were reduced by 28% and 34%, respectively, as compared with non-smokers.

In this study, we did not find significant changes in the plasma levels of α-tocopherol/total lipid, ascorbate, and lipid peroxides in smokers younger than 35. But this is not the case in smokers older than 35 (Table 2). Although α-tocopherol and ascorbate can be obtained from the diet or vitamin supplements, the plasma levels of the two antioxidants are maintained, at least to some extent, by the endogenous regeneration systems. Regeneration of ascorbate by uric acid and of α-tocopherol by ascorbate take place in the lag phase of oxidation of human plasma [22]. However, this regeneration in the biological setting needs to be further coupled to non-radical reducing systems such as reduced glutathione (GSH)-oxidized glutathione (GSSG), dihydrolipoate-lipoate, NADPH-$NADP^+$, and NADH-NAD^+ [23].

Our findings indicate that the activities and capacities of these antioxidants-regeneration systems in young smokers are sufficient for the effective disposal of ROS and free radicals generated in smoke. This explanation is supported by our previous study which showed that young smokers, but not aged smokers, had significantly higher levels of blood glutathione [10]. As age increases, the activity of such regeneration systems may be decreased causing the depletion of plasma α-tocopherol and ascorbate in elderly smokers.

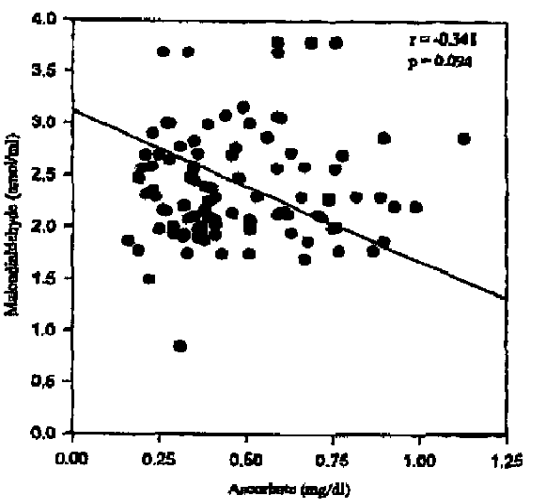

Fig. 2. Plasma level of malonialdehyde is negatively correlated with plasma ascorbate concentration. There are 103 data sets from 39 male smokers and 64 male non-smokers in the scattered plot. The *P*-value was calculated by partial correlation analysis under age control. *r*, sample correlation coefficient.

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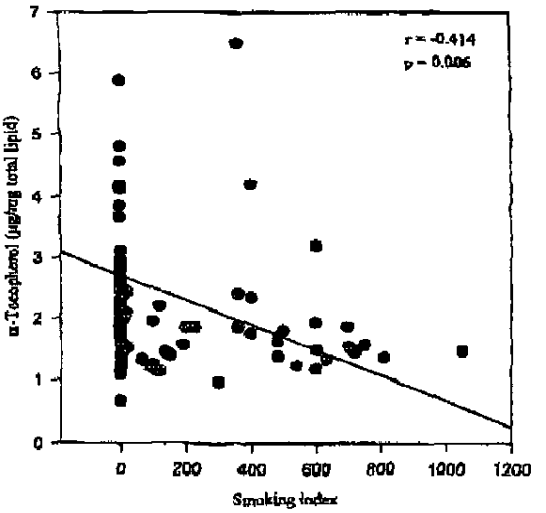

Fig. 3. Negative correlation between plasma level of α-tocopherol and smoking index. There are 103 data sets from 39 male smokers and 64 male non-smokers in the scattered plot. Smoking index is defined as the number of cigarettes smoked per day × years of smoking. The *P*-value was calculated by partial correlation analysis under age control. *r*, sample correlation coefficient.

In this study, we found that between 35 and 45 years of age smokers experience a significant reduction of plasma α-tocopherol and ascorbate upon cigarette smoking. Moreover, we noted a significant increase in the plasma level of α-tocopherol of non-smokers between 35 and 45 years of age, but the elevation was not sustained in smokers especially when their ages reached above 45 (Table 2). This phenomenon of age-dependent elevation of plasma α-tocopherol is very unlikely to be caused solely by an increased regeneration of α-tocopherol in non-smokers. We tend to believe that depletion of plasma α-tocopherol in aging smokers is due to both increased consumption and decreased absorption/regeneration of α-tocopherol in the human body as a whole. In contrast, a compensatory induction (due to an ever-increasing need) of higher α-tocopherol absorption and regeneration and other factors including decreased physical activity and increased intake of antioxidants may be all involved in the age-dependent increase of plasma level of α-tocopherol in non-smokers.

Retinol plays an important role in growth, proliferation and differentiation of epithelial cells, and maintenance of normal visual and reproductive functions of the human [23]. Helen and Vijayammal [9] reported that lipid peroxidation induced by cigarette smoke can be prevented by supplementation of smokers with retinol via its antioxidant activity. Nevertheless, they failed to show that there is a significant decline of retinol in the plasma of smokers. In this study, we did not find any difference in the plasma levels of retinol between smokers and non-smokers. This

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may indicate that retinol is not a main antioxidant involved in the relief of smoking-elicited oxidative stress. Eiserich et al. [24] reported that exposure of blood plasma, a model for respiratory tract lining fluids, to gas-phase cigarette smoke causes depletion of uric acid and glutathione. However, we did not find any reduction in plasma level of uric acid in smokers examined in this study. This may suggest that uric acid is continuously generated in the human body from the metabolism of purine-containing food, and thereby minimizes the difference in plasma level of uric acid between smokers and non-smokers.

Smoking-elicited oxidative stress may enhance lipid peroxidation through the attack of membrane lipids by ROS and organic free radicals. It is thus not surprising to find that aging smokers have increased levels of lipid peroxides in plasma, which may be due to an age-dependent decline in the activity and capacity of free radical scavengers and antioxidants in the blood circulation. In this study, we found that the smoking-induced decrease in the levels of plasma ascorbate and α-tocopherol was correlated with the increase of plasma level of MDA in both smokers and non-smokers. However, only the plasma level of α-tocopherol was found to significantly correlate, in a negative manner, with the smoking index (Fig. 3). This finding may suggest that α-tocopherol is the major lipid-soluble chain-breaking antioxidant in smokers, which prevents plasma lipids from oxidative damage caused by smoking-generated ROS and free radicals.

In conclusion, our findings suggest that adequate levels of α-tocopherol and ascorbate provide antioxidant protection of the plasma of smokers from oxidative damage by smoking-mediated ROS and free radicals in young smokers. However, the antioxidant defense system formed by the coordinated action of α-tocopherol and ascorbate or glutathione is probably overwhelmed in elderly smokers by the long-standing oxidative stress elicited by cigarette smoking.

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References

[1] W.A. Pryor, K. Stone, Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite, Ann. New York Acad. Sci. 686 (1993) 12–27.

[2] R. Cueto, W.A. Pryor, Cigarette smoke chemistry: conversion of nitric oxide to nitrogen dioxide and reactions of nitrogen oxides with other smoke components as studied by Fourier transform infrared spectroscopy, Vibrat. Spectr. 7 (1994) 97–111.

[3] B. Frei, T.M. Forte, B.N. Ames, C.E. Cross, Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma: protective effects of ascorbate, Biochem. J. 277 (1991) 133–138.

PM3006722938

Source: https://www.industrydocuments.ucsf.edu/docs/pybj0001

[4] A.Z. Reznick, C.E. Cross, M.L. Hu, et al., Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation, Biochem. J. 286 (1992) 607–611.
[5] G.A. Pascoe, K. Olafsdottir, D.J. Reed, Vitamin E protection against chemical-induced cell injury. I. Maintenance of cellular protein thiols as a cytoprotective mechanism, Arch. Biochem. Biophys. 256 (1987) 150–158.
[6] T.T. Nguyen, C.S. Cox, D.L. Traber, H. Gasser, H. Redl, G. Schlag, D.N. Herndon, Free radical activity and loss of plasma antioxidants, vitamin E and sulfhydryl groups in patients with burns, J. Burn Care Rehabil. 14 (1993) 602–609.
[7] B. Frei, L. England, B.N. Ames, Ascorbate is an outstanding antioxidant in human blood plasma, Proc. Natl. Acad. Sci. USA 86 (1989) 6377–6381.
[8] B.N. Ames, R. Cathcart, E. Schwiers, P. Hochstein, Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis, Proc. Natl. Acad. Sci. USA 78 (1981) 6858–6862.
[9] A. Helen, P.L. Vijayammal, Effect of vitamin A supplementation on cigarette smoke-induced lipid peroxidation, Vet. Hum. Toxicol. 39 (1997) 18–21.
[10] C.S. Liu, Y.H. Wei, Alteration of blood thiol-group-related antioxidants in aging smokers, Environ. Res. (1998) in press.
[11] C.S. Liu, S.H. Kao, Y.H. Wei, Smoking-associated mitochondrial DNA mutations in the human hair follicles, Environ. Mol. Mutagen. 30 (1997) 47–55.
[12] G.L. Catignani, J.G. Bieri, Simultaneous determination of retinol and α-tocopherol in serum or plasma by liquid chromatography, Clin. Chem. 29 (1983) 708–712.
[13] C.S. Frings, R.T. Dunn, A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction, Am. J. Clin. Pathol. 54 (1970) 89–91.
[14] A. Kyaw, A simple colorimetric method for ascorbate determination in blood plasma, Clin. Chim. Acta 86 (1978) 153–157.
[15] J.C. Doussaet, M. Trouilh, M.J. Foglietti, Plasma malondialdehyde levels during myocardial infarction, Clin. Chim. Acta 129 (1983) 319–322.
[16] E.R. Pacht, H. Kuseki, J.R. Mohammed, D.G. Cornwell, W.B. Davis, Deficiency of vitamin E in the alveolar fluid of cigarette smokers: influence of alveolar macrophage cytotoxicity, J. Clin. Invest. 77 (1986) 789–796.
[17] E. Hoshino, R. Shariff, A. van Gossum, J.P. Allard, C. Pichard, R. Kurian, K.N. Jeejeebhoy, Vitamin E suppresses increased lipid peroxidation in cigarette smokers, J. Parent. Enter. Nutr. 14 (1990) 300–305.
[18] K. Marangon, B. Herbeth, E. Lecomte, A. Paul-Dauphin, P. Grolier, Y. Chanconelle, Y. Artur, G. Siest, Diet, antioxidant status, and smoking habits in French men, Am. J. Clin. Nutr. 67 (1998) 231–239.
[19] I.F. Pons, A. Frigola Canoves, I. Rubio-Aliaga, Vitamin C status of adults living in Valencia, Spain. Influence of non-nutritional factors on plasma vitamin C, Int. J. Vitam. Nutr. Res. 67 (1997) 255–261.
[20] L.H. Strauss, P. Scheer, On the influence of nicotine on the concentration of vitamin C, Z. Vitaminforsch. 9 (1939) 39–48.
[21] A.A. Albanese, E.H. Wein, L.A. Matu, An improved method for determination of leucocyte and plasma ascorbate of man with application to studies on nutritional needs and effects of cigarette smoking, Nutr. Rep. Int. 12 (1975) 271–289.
[22] B. Karten, U. Beisiegel, G. Gercken, A. Kontush, Mechanisms of lipid peroxidation in human blood plasma: a kinetic approach, Chem. Phys. Lipids 88 (1997) 83–96.
[23] H. Sies, W. Stahl, Vitamins E and C, beta-carotene, and other carotenoids as antioxidants, Am. J. Clin. Nutr. 62 (6 Suppl.) (1995) 1315S–1321S.
[24] J.P. Eiserich, A. van der Vliet, G.J. Handelman, B. Halliwell, C.E. Cross, Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction, Am. J. Clin. Nutr. 62 (6 Suppl) (1995) 1490S–1500S.

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